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NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

*Technical Memorandum 33-490*

*Flow Dilution Effect on Blood  
Coagulation In Vivo*

*Chia-lun Hu*

JET PROPULSION LABORATORY  
CALIFORNIA INSTITUTE OF TECHNOLOGY  
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National Aeronautics and Space Administration

## PREFACE

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## ABSTRACT

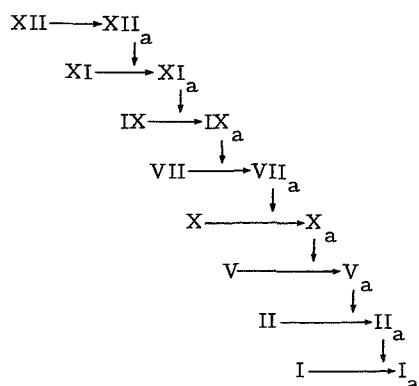
Self-regulation is a major characteristic pertaining to in vivo blood coagulation. In vivo coagulation is a set of enzyme reactions that are inhomogeneous in space. The inhomogeneity is maintained by the dilution of the blood flow in vivo. Because of this inhomogeneity, the chemical rate equations should be modified by fluid-dynamic (or flow-dilution) change and diffusion change. This report proposes a simple model of enzyme reactions and emphasizes the flow-dilution change of the reactions. First, the complex reactions in blood coagulation are discussed and weighed. Second, two controlling chemical reactions, the prothrombin-to-thrombin conversion and the inactivation of thrombin, both of which have significant positive and negative feedbacks, are selected. It is seen then, that the reaction rates of both these reactions will decrease as thrombin concentration is decreased by flow dilution. But the positive rate decreases more (because of the autocatalyzation of the prothrombin-thrombin conversion) than the negative rate. Therefore when flow dilution increases, the overall reaction direction can be switched from the positive (procoagulative) direction to the negative (anticoagulative) direction; thus the in vivo coagulation is regulated and confined. This physical picture is analytically investigated by solving the modified Michaelis-Menton's enzyme rate equations. The effect of varying the antithrombin concentration is also investigated. The background and the physics of this analysis are extensively discussed.



## I. INTRODUCTION

Blood coagulation is a very complex phenomenon. It involves more than a dozen complex enzyme reactions. These reactions may be initiated by various mechanisms, for example, by contact with a foreign surface, by contact with materials from broken tissues, broken blood cells, or broken platelets, by imbalance of plasma factors, and by unknown defects in the circulating system. Many texts (Refs. 1-5) and thousands of papers (Ref. 6) on the subject of blood coagulation have been published since the last century. In the last twenty years, however, a major portion of the literature in this field has been devoted to the identification of enzyme reactions involved in blood coagulation in vitro. [Ferguson recently published a long review article on this, which includes 250 references (Ref. 7).] From these in vitro studies, at least two major theories have been proposed to date to explain the enzyme reactions in the blood coagulation process. One is the cascade theory (Ref. 8) or the waterfall theory (Ref. 9), which is represented in the following diagram:

Activating mechanisms



All the Roman numbers here are the international symbols for the preactivated and activated (with subscript a) plasma factors: II is the prothrombin, II<sub>a</sub> is the thrombin, I is the fibrinogen, I<sub>a</sub> is the fibrin. In this diagram, the product of every reaction step is assumed to be the catalyst for the next step. This theory does not show the inactivating mechanism of thrombin and the autocatalyzing effect of the prothrombin-to-thrombin reaction. Also, cross (or feedback) paths of many reactions are not indicated in the cascade.

The second major theory was proposed by Seegers (Ref. 10). He proposed a set of more complicated enzyme pathways as shown in Fig. 1. It includes some feedback reactions, but the weight (probability) of each pathway is not fully discussed.

From the above short sketch, it is seen that the theories actually propose different sets of chemical pathways that the enzymes in the blood will follow when coagulation proceeds. The influence of blood flow on chemical kinetics is not discussed in these theories. In general, extensive analytical studies seem to occupy only a small portion of the contemporary literature of blood coagulation (Refs. 11 and 12). Particularly, to the author's knowledge, the self-regulatory phenomenon of in vivo blood coagulation has hardly been explored analytically.

Self-regulation of blood coagulation in vivo is familiar to us as a phenomenon. [It is also referred to as the ability of maintaining blood fluidity in vivo (Ref. 13).] As we know, without anti-coagulant, the blood coagulates easily in vitro. The coagulation, once initiated, will spread to the whole volume of the blood. But, in a healthy man, in vivo coagulation does not spread easily through his circulating system even if the coagulation is initiated by the same agent (e. g., contact with

foreign surface) in vitro and in vivo. Clearly, there must be some self-regulatory mechanism in vivo that is not effective in vitro. To the author's knowledge, there are at least two recognized factors that might account for the difference between these two cases of coagulation.

The first is the flow dilution effect, which is operative when clotting is initiated on the wall of a vessel that is not completely severed. The activated enzymes in the in vivo blood will be diluted by circulating blood all the time. As explained later, these diluted enzymes (procoagulants) will eventually give way to the anticoagulants that normally exist with constant concentrations in the blood. Therefore, the coagulation, once initiated, will eventually be confined in the in vivo flowing blood. This dilution control of the in vivo coagulation has been mentioned in a general way by MacFarlane-Smith (Ref. 14), Burton (Ref. 15), and Waugh (Ref. 16).

The second factor concerns the functions of the blood vessels.<sup>1</sup> For example, nine different kinds of blood vessels have been found to have anticoagulants in their walls (Ref. 17). These anticoagulants do not exist in blood in vitro. But in vivo, it is doubtful that they exert a major effect since it has been shown that the blood can still coagulate easily (without regulation) in isolated venous segments (Ref. 18). On the other hand, the flow dilution effect seems to be commonly recognized as the regulatory mechanism although it has not been analytically examined.

This report discusses a kinetic model of blood coagulation that takes the flow dilution effect into account. It is a model of modified Michaelis-Menton's enzyme kinetics applied to blood coagulation.

The literature of the kinetics of clotting enzymes can be divided into two groups. The first group is the major one that deals with the in vitro identification and rate determination of enzyme pathways in blood coagulation. The second group reports more analytical results, for example, calculations of reaction constants, in vitro studies of the variations of clotting time with respect to enzyme concentrations, and investigations of the kinetics of some simplified chain reactions (Refs. 19-39). In general, however, it is to be noted that most of the papers in the second group do not take into account the physical differences between the in vivo and in vitro states of the blood. For example, most analyses follow from the Michaelis-Menton's model, which, without modification, is a kinetic model for stationary and homogeneous enzyme reactions only. This is not the case for the in vivo coagulation reactions that are both nonstationary (flowing) and inhomogeneous. Also, most analyses do not include a complete transient-asymptotic solution of the rate equations. The complete transient-asymptotic solution is necessary for studying the buildup and confinement of in vivo coagulation. In contrast to all these investigations, the goal of this report is to present the physical complexity involved in the in vivo case and to draw a much simplified model from it such that it can be shown analytically that the flow dilution alone is sufficient to explain the self-regulatory phenomenon of in vivo coagulation.

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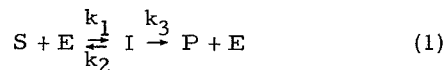
<sup>1</sup>One may think that chemical reactions may take place across the vessel wall such that the anticoagulation feedback may come from outside the vessel wall by permeating the enzyme molecules through the wall. But this is very unlikely, because the permeating rate should be much too slow to match the procoagulation reactions happening inside the wall.

## II. THE MODEL

Three major assumptions of the present model are discussed in detail in this section.

### A. Assumption 1: A Modified Michaelis-Menton's Model of Enzyme Reactions Is Assumed

Michaelis-Menton's model of enzyme reaction is a homogeneous, two-stage chemical reaction as shown in the following:



where S, E, I, P represent, respectively, the molecules of substrate, enzyme, intermediate, and product. In this model, it is assumed that the concentration of the intermediate I reaches a steady state very fast as compared with other changes in the reaction. That is,

$$\frac{\partial I}{\partial t} = 0 \quad (2)$$

is assumed valid during the main course of the reaction. With this assumption, the rate of producing P can thus be derived, in terms of the rate constants and the initial concentrations, in a much simplified way. However, when this model is applied to in vivo coagulation reactions a modification must be added to the rate equations; this modification is the flow dilution effect of the activated enzymes. The mechanism and modeling of this flow dilution effect will be discussed in Assumption 3.

### B. Assumption 2: The Main Controlling Steps in the Chemical Reactions of Coagulation Are the Prothrombin-to-Thrombin (P-T) Conversion and the Inactivation of Thrombin T by Antithrombin A.

The P-T conversion is the step that immediately proceeds the fibrinogen-to-fibrin (F-fn) reaction, which is the final chemical event in forming a clot. The product of the P-T conversion, i. e., T, is the activating enzyme for the F-fn reaction. It appears that among all the reactions leading to clot formation, only P-T conversion and the inactivation of T have appreciable positive and negative feedback mechanisms. The positive feedback is due to the autocatalysis of the P-T reaction. That is, T is an enzyme that can accelerate the P-T reaction. Therefore, the more T produced, the faster the acceleration of the P-T reaction. The negative feedback is due to the function of antithrombin A normally existing in the plasma. When T is produced, A will react with it and turn it back to the inactivated form. That is, A can destroy the active T and consequently slow down the coagulation.

Next, we shall investigate the relation between the variation of T and the clot forming criterion. The F-fn reaction usually is considered as consisting of three steps (Ref. 40). First,  $F \xrightarrow{T} f + R$ , where f is the fibrin monomer and R is a residual. Second,  $m f \rightarrow fm$ , where fm is a partially polymerized molecule. Finally,  $n fm \rightarrow fn$ , where fn is the completely polymerized clot. T is an enzyme in the first step and is not consumed chemically during the reaction (adsorption of T by the clot will be discussed shortly); yet without T, the F-fn reaction will stop immediately because all the three steps in the reaction are not autocatalyzed reactions. Therefore, to complete the polymerization of the clot, a certain amount of T must be present long enough to produce a concentration of fibrin monomers sufficient for polymerization. If the concentration of T at a flowing point appears only in a very short period of time, then, except for some isolated fm molecules possibly formed, the clot will not be formed at that point. These fm molecules will be diluted in the blood stream and fibrinolized later by protamine. Consequently, a criterion for a point in the blood flowing closely by an in vivo clot to coagulate and to join the clot is the following:

The quantity

$$B \equiv \int_0^{\tau} T \, dt \quad (\text{footnote 2})$$

for that flowing point must be greater than a critical value C. Here T is the thrombin concentration at that flowing point and  $\tau$  is the time required for that point to flow by the clot surface. (3)

When B at a flowing point under consideration is greater than C, there exists enough T in a certain amount of time or there exists a certain amount of T in an enough time for complete polymerization at that point when it passes by the in vivo clot surface. Therefore, that point will clot and stick to the clot surface. Consequently, the in vivo clot grows.

On the other hand, when B is smaller than C, that point will not complete the polymerization during its course flowing by the clot. Therefore, that point, even if it polymerizes later, will not stick to the clot. Thus, the in vivo clot is confined.

Now we shall discuss the reactions before the P-T reaction and those after the clot is formed. The reactions before the P-T reaction have no appreciable feedback mechanism. If any of these

<sup>2</sup>Loosely speaking, B is the "product" of the thrombin concentration and the effective reaction time for a point flowing by the clot just formed.

reactions is initiated by any means, it will converge to the P-T reaction before the clot is formed. Therefore, even if all the enzymes at some point are activated but the P-T reaction is still confined by some mechanism (e.g., by injecting antithrombin into the vessel) a clot still will not be formed, and all the activated enzymes will be proteolyzed later in the blood stream. For the reactions after the clot is formed, there also is no important controlling effects to the clot, because once the clot is formed, it cannot be destroyed by immediately next reactions. For example, the adsorption of T by a fibrin clot can take place only after the polymerization is completed, while the confinement of the clot is determined [according to Relation (3)] before the completion of the polymerization. Therefore, the adsorption of T by the clot will not have significant feedback effect on forming the clot. Another reaction after clotting is fibrinolysis. This is a proteolytic process that dissolves the clot by means of proteolytic enzymes. Its rate should be very slow to give any immediate effects after the clot is formed.

The use of T in F-fn reaction will also affect the rates of the two controlling reactions. But its effect should be small. The reason is the following: T is a catalyzer in the F-fn reaction; the lifetime of the intermediate formed by a catalyst molecule and a substrate molecule is usually very short, i. e., the intermediate will usually decompose to a product molecule and a catalyst molecule very fast. Therefore, to a first approximation, the decreasing of "free" T concentration due to the engagement of T molecules in the catalyzation of the F-fn reaction can be neglected as compared to the creating rate and destroying rate of T due to both controlling reactions.

Finally, the platelet also plays an important role in the blood coagulation process by initiating the chain reactions there, and by accelerating the P-T reaction without appreciable feedback effect (Refs. 41 and 42). In this report, the effects of platelets and other nonfeedback procoagulants such as Ac-globulin, vitamin K, and calcium ions are assumed as part of the reaction constant of the P-T reaction.

#### C. Assumption 3: A Much Simplified Dilution Model Is Assumed

In vivo coagulation is a set of inhomogeneous chemical reactions affected significantly by dilution effect. "Inhomogeneous" here means that the concentrations of reactants and products are not homogeneous in space, and initiation mechanisms are not homogeneously introduced to the blood. For in vitro experiments, the homogeneity can be reached in a short time either by self-diffusion of molecules or by shaking the test tube. But for in vivo blood coagulation, the homogeneity can hardly be reached during the whole course of reactions because of the constant dilution<sup>3</sup> of the activated enzymes due to continuous wash of the flowing

blood. For an inhomogeneous chemical reaction, the chemical changes should vary from "point"-to-"point" in space. The word "point" here actually means a macroscopic "point", not a geometrical point. It is a sphere in the inhomogeneous medium. This sphere is small enough so that chemical concentrations inside can be considered homogeneously distributed in space all the time, but large enough so that the chemical reactions inside will not get down to molecular scale with large inhomogeneity in space. This sphere is tentatively called the "sphere of homogeneity". In the following, whenever the phrase "a (flowing) point" is mentioned, it means actually "a (flowing) sphere of homogeneity". For an inhomogeneous stationary fluid, this sphere is stationary in space, and the rate equation of any chemical reaction inside the sphere would be modified only by a diffusion change as a result of the inhomogeneity. For an inhomogeneous moving fluid, however, the sphere of homogeneity moves with the macroscopic particle velocity of the fluid, because chemical reactions in the fluid are "following-the-particle" (in the sense of fluid-dynamic terms) or "following-the-point" reactions. For this flowing fluid, additional corrections due to flow dilution must be made for the rate equations. Strictly speaking, the form of these flow dilution terms should be determined by the statistical fluid mechanics of the fluid. This, however, would certainly make the rate equations excessively complicated to solve as well as to formulate without much gain in physical implications. Therefore, in this report, it is assumed only that the flow dilution will give an additional change of the concentration of a certain newly produced chemical (e.g., thrombin) at a flowing point according to the following relation:

$$\left(\frac{\partial M}{\partial t}\right)_{\text{due to flow dilution}} = -\alpha M \quad (4)$$

where M is the concentration of the newly produced chemical in mole/liter and the proportionality constant  $\alpha$  has the following property:

$\alpha$  is an increasing function of the inhomogeneity (e.g., the gradient of the velocity) at the flowing point and  $\alpha$  is zero when the fluid is stationary. (5)

Relation (4) means that the more the molecules aggregate at a point, the more the molecules will be washed away per unit time by the flow dilution, while Relation (5) is just a result of the fact that the larger the inhomogeneity, the larger the rate of flow dilution, and no flow dilution can occur when the fluid is stationary.<sup>4</sup> Usually  $\alpha$  should increase as the clot grows in vivo. For example, if an in vivo clot grows in a large vessel, the velocity of the clot is always zero, while the velocity of the blood increases from zero near the vessel wall to a maximum on the axis of the vessel. Therefore, the dilution effect should grow larger and larger when the clot grows deeper and deeper into the blood stream of a large

<sup>3</sup>It is to be noted that the dilution effect here is to be distinguished from the dilution of the plasma mentioned in many in vitro experiments in the literature (Refs. 19-36). The latter is a homogeneous dilution of all molecules by adding water or other solvents to the plasma, while the former is an inhomogeneous dilution of the activated molecules only by flowing blood.

<sup>4</sup>Section V has more discussion on this model of the dilution effects.

vessel. For stationary blood, the pure diffusion dilution is responsible for spreading the coagulation. But for flowing blood, the pure diffusion dilution should be negligible as compared with the flow dilution. Therefore, in the following formulation of in vivo coagulation, pure diffusion effect is neglected, and only Relation (4) is added to the "following-the-point" chemical rate equations.

Next, we shall discuss the effect of flow dilution on prothrombin concentration. Prothrombin P is an enzyme normally existing in the blood with constant concentration. When it participates in the  $P \rightarrow T$  reaction taking place locally at the perturbed region, its concentration will decrease locally there. But the blood flow tends to mix the perturbed blood with the unperturbed blood so as to keep the P concentration in the perturbed region normal. Therefore, the effect of blood flow on the prothrombin that normally exists in the blood is a "refilling" increase of the concentration in contrast to the dilution decrease of the concentrations for those enzymes not normally existing in the blood.

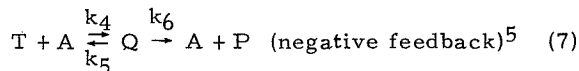
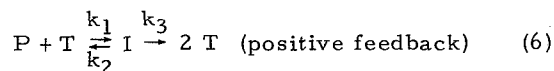
Finally, we shall discuss the initial condition for the blood flow model assumed here.

When the coagulation proceeds in vivo, the thrombin molecules will diffuse from the clot already formed and mix immediately with the blood flowing by. The "taking-up" of these thrombin molecules by a flowing point in the blood is a very complicated phenomenon. It is a continuous process in time and space, and it depends very much on the path of the flowing point. Its exact behavior, again, should be determined by fluid dynamics and diffusion mechanics. But to a first approximation, we can assume that any flowing point entering the perturbed region will take up an amount of thrombin that has an initial concentration  $T_0$ . If there were no chemical reactions and no flow dilution, this  $T_0$  would be constant along its path flowing by the clot. But when flow dilution and chemical reactions are taken into account as analyzed in Section III, this  $T_0$  is then the initial concentration of T taken up by a flowing point entering the perturbed region. The concentration of T at this point will then vary according to the analysis given in Section III.

With these three assumptions of the model in mind, we are going to formulate the chemical rate equations and to solve them in the next section.

### III. RATE EQUATIONS AND SOLUTION

Following the Michaelis-Menton's model, Reaction (1), we can write the enzyme reactions of the controlling steps taking place at a flowing point as:



where P, T, A, I, Q are, respectively, the molecules of prothrombin, thrombin, antithrombin, and intermediates. The "following-the-point" rate equations of the chemicals involved in the above reactions are:

$$\frac{dT}{dt} = -k_1PT + (k_2 + 2k_3)I - k_4AT + k_5Q - \alpha T \quad (8)$$

$$\frac{dI}{dt} = k_1PT - (k_2 + k_3)I - \alpha I \quad (9)$$

$$\frac{dQ}{dt} = k_4AT - (k_5 + k_6)Q - \alpha Q \quad (10)$$

All T, P, I, Q, A molecules are now concentrations expressed in mole/liter. The last terms in these equations are the flow dilution terms given by Equation (4);  $\alpha$  has the property given by Relation (5). The rate equations of other chemicals are not used in the following calculation because of Michaelis-Menton's assumption.

Now the conservation of mass will be considered. If the lifetimes of the intermediates I and Q in Reactions (6) and (7) are quite short (this is the usual case for enzyme reactions), if T is not occupied by F<sub>1</sub>T<sub>2</sub> reaction too long as discussed in Assumption 2, and if the dilution effect on T is compensated by the "refilling" effect on P (see Assumption 3), then we should have, for all the time,

$$P + T = P_0 = \text{constant} \quad (11)$$

Finally, the initial condition of the reactions at a flowing point under consideration as discussed at the end of Assumption 3 is

$$T = T_0 \quad \text{at } t = 0 \quad (12)$$

At this point, the mathematical formulation is complete and we can solve the problem as follows.

Applying Equation (2), we see that  $dI/dt = dQ/dt = 0$ . Thus Q and I can be solved from Equations (9) and (10) algebraically. Substituting Q and I into Equation (8) and eliminating P by Equation (11), we have

$$\frac{dT}{dt} = \theta[\psi - T]T \quad (13)$$

where

$$\left. \begin{aligned} \theta &= \frac{k_1(k_3 - \alpha)}{\alpha + k_2 + k_3} \\ \psi &= P_0 - \left[ \frac{k_4(k_6 + \alpha)}{k_5 + k_6 + \alpha} A + \alpha \right] / \theta \end{aligned} \right\} \quad (14)$$

The general solution of Equation (13) with the initial condition of Equation (12) is easily obtained as

$$T = \frac{\psi}{1 - \frac{T_0 - \psi}{T_0} e^{-\theta T_0 t}}$$

However, the variations of the T-t curves with respect to the changes of  $\alpha$  and A (or the flow dilution rate and the antithrombin concentration) are quite complicated. The study of these variations and their significances are discussed in the next section and Appendices A and B.

<sup>5</sup>If the negative "feedback" reaction is  $T + A \rightarrow X$  instead of Reaction (7), then the mathematics should be modified but the result and the physical consequences should not change because the destroying rate of T from the new reaction is still linearly proportional to T. Therefore, the dilution decrease of T will still have less effect on this new negative reaction than on the positive (autocatalytic) Reaction (6). Consequently, the procoagulation tendency can still be switched to the anticoagulation tendency when dilution rate increases. The reason that Reaction (7) is proposed here is that there must be a return path for T to P, otherwise the in vivo blood is not in a stable state — any small ignition will change P permanently.

#### IV. ANALYSIS AND INTERPRETATION OF THE RESULT

The variations of the T-t curves with respect to  $\alpha$  and A are studied analytically in Appendices A and B, respectively. The result follows.

##### A. The Variation of the T-t Curves with Respect to $\alpha$ or the Flow Dilution Rate

When A in the blood is kept at a constant concentration  $A_0$ , this variation is shown in Fig. 2. Several points are to be noted here.

- (1) Each of these curves will approach an asymptotic level  $T_\infty$  monotonically when t approaches infinity, and

$$T_\infty = \begin{cases} \psi(\alpha) & \text{when } \psi(\alpha) \geq 0, \text{ and } \theta(\alpha) > 0 \\ 0 & \text{when } \psi(\alpha) \theta(\alpha) \leq 0 \end{cases} \quad (16)$$

$\psi(\alpha)$  is a function of  $\alpha$  given by Equation (14). It is a monotonically decreasing function of  $\alpha$ .  $\alpha_b$  shown in the figure is the root of the equation  $\psi(\alpha) = 0$ . The curve with  $\alpha = \alpha_b$  is the boundary curve between the curves with  $T_\infty \neq 0$  and with  $T_\infty = 0$ .

- (2) The steepness of the curve will increase (or the slope will decrease) as  $\alpha$  increases. Theoretically, when  $\alpha$  increases to infinity, the transient thrombin concentration will be a step-down function in time as shown.

Now we can see that no matter how large  $\tau$  is, there is always a curve with  $\alpha = \alpha_c$  such that the relation  $B = C$  [B is the quantity defined in Relation (3)] can be satisfied. For the curves below this curve ( $\alpha > \alpha_c$ ),  $B < C$  and for those above this curve ( $\alpha < \alpha_c$ ),  $B > C$ . The reason for this is that B decreases with  $\alpha$  when  $\tau$  is fixed and B approaches zero when  $\alpha$  approaches infinity. (Integration of a stepdown function as shown in Fig. 2 as  $\alpha \rightarrow \infty$  is always zero). Therefore, if the flow dilution can be increased indefinitely, there always exists a critical flow dilution rate  $\alpha_c$  such that beyond which no clotting is possible.

- (3) A comparison between the curves in Fig. 2 (for which both dilution and chemical reactions are in action) and the curves due to flow dilution alone has the following properties: for small dilution rates ( $\alpha$  small), the decreasing rate of T due to both mechanisms is slower than that due to dilution alone; for large dilution rates ( $\alpha$  large), the situation reverses. The proof of this statement follows.

According to Assumption 3 and Equation (4), the rate of change of T at a flowing point due to dilution alone will

be  $dT/dt = -\alpha T$ . The solution of this equation is

$$T = T_0 e^{-\alpha t} \quad (17)$$

It is seen that T always approaches zero when t approaches infinity. The decreasing rate of T in Equation (17) is larger than that of the curves in Fig. 2 when  $\alpha$  is small, because the latter will not approach zero and some of them may even "increase" with respect to time. For large  $\alpha$ , then, as shown in Appendix C, the solution of T due to both mechanisms can be approximated by

$$T \approx T_0 e^{\theta \psi t} \equiv T_0 e^{-|\theta \psi| t} \quad (18)$$

where

$$\theta < 0, \psi > 0$$

and, from Equation (14),

$$|\theta \psi| = k_1 P_0 \frac{\alpha - k_3}{\alpha + k_2 + k_3} + \frac{k_4 (k_6 + \alpha) A}{\alpha + k_5 + k_6} + \alpha$$

This  $|\theta \psi|$  is larger than  $\alpha$ . Therefore, when  $\alpha$  is large, T in Equation (18) decreases faster than that due to dilution alone (Equation 17), QED.

##### B. The Variation of the T-t Curves with Respect to A or the Antithrombin Concentration

This will be quite similar to that when  $\alpha$  is varied. Schematically, if all the  $\alpha$ 's in Fig. 2 are replaced by A, we then obtain a set of T-t curves with A as the running parameter. Consequently, we see that, for a fixed  $\alpha$ , there also exists a fixed antithrombin concentration,  $A_c$ , such that when  $A > A_c$ , no clot will be formed at the flowing point under consideration, and when  $A < A_c$ , clot will be established there.

##### C. The Case Where $T_0 = 0$

When  $T_0 = 0$ , from Equation (15), T will be equal to zero all the time.

From the properties discussed in Subsections A, B, and C, some important physical consequences can be drawn immediately.

- (1) No matter whether the blood is flowing or stationary, the blood cannot clot by itself without some thrombin present or produced in the blood. This follows from the statement in Subsection C. If by any means, some thrombin is produced in the blood, then the coagulation may take place, may spread indefinitely, or may be

confined locally, depending on the situation of the blood.

- (2) A clot can easily develop in a stationary blood ( $\alpha = 0$ ), but not in a flowing blood ( $\alpha \neq 0$ ). If thrombin is present at any point in a stationary blood due to any activating mechanism introduced to that point, or due to the arrival of the thrombin molecules diffusing from the adjacent points, that point will clot and the clot will spread by diffusion of the residual thrombin molecules. This is because the  $\alpha = 0$  curve in Fig. 2 is above the critical curve with  $\alpha = \alpha_c$ , therefore, the clot will develop. On the other hand, when blood is flowing in a vessel, the more the clot grows in the flowing blood the more the dilution will be as discussed in Assumption 3. Above a critical dilution rate ( $\alpha \geq \alpha_c$ ), the blood flowing by the clot will stop clotting because B will be smaller than C when  $\alpha \geq \alpha_c$ . Therefore, by Relation (3), these points will not clot and thus the clot is confined.
- (3) Thrombin molecules are produced or destroyed (inactivated) chemically (not just diluted) depending on whether the flow dilution is small or large. As proved for low dilution rate in point (3) of subsection A of this Section, the actual decreasing rate of T (Fig. 2) is slower than that due to dilution alone; for high dilution rate, the situation reverses. Therefore, thrombin molecules are actually produced at the flowing point under consideration when the dilution there is small, and destroyed when the dilution is large. This can be seen more directly from the two major chemical reactions listed in relations (6) and (7). The prothrombin-thrombin reaction is an autocatalyzed reaction. Loosely speaking, the producing rate of T due to this reaction is proportional to the square of T (thrombin concentration). On the other hand, the A-T reaction (inactivation of thrombin) is not an autocatalyzed reaction. Generally, the destroying (inactivating) rate of T is proportional to T only. Therefore, the loss of T due to dilution will cause the producing rate to decrease more than the destroying rate. Consequently, the reaction direction is switched from producing T to destroying T when flow dilution increases.
- (4) If antithrombin is added to the blood, the effect will be similar to that of increasing the dilution rate. This follows immediately from subsection B of this Section.



## V. DISCUSSION

### A. Dilution Effect on the In Vitro Flow

The in vitro flow dilution must be a real dilution to allow the self-regulation phenomenon to occur.

Some in vitro flowing-blood experiments have shown artificial thrombosis or the fast clotting effect (without self-regulation) (Refs. 43-51). This seems to be a violation of the principal result described in this report. But a careful study of these experiments shows that the activating surfaces in these experiments are so large that the activated enzymes are almost everywhere. Consequently not much dilution effect can be expected even where the blood flows rapidly in these systems. Among these experimental investigations, Dentinfass' reports on the viscometer experiments are particularly interesting.

Dentinfass (Refs. 45-51) has done a series of in vitro experiments showing that the larger the shearing rate of the blood flow in vitro, the shorter the clotting time. This, at a first glance, seems to be a contradiction of what this report proposes. However, a careful examination of his experimental procedures and apparatus reveals that there are many differences between his in vitro system and the in vivo system. First, the blood in his experiments is put between two coaxial cones of a viscometer with the outer one rotated at a controlled speed. The surfaces of the cones are not specially treated with anticoagulants. They are the foreign activating surfaces to the blood. Therefore, when the blood rotates as the outer cone rotates, not much dilution effect can be expected since every circumferential point on the path of the blood movement is equally activated. Second, the rotation starts at zero velocity, and, at the beginning, the blood cells are distributed uniformly in the cross section of the flow. Therefore, the blood cells in the boundary layer will be subject to large shearing stresses as rotating speed increases. It is very likely that many blood cells are broken during the starting period of the experiments. A recent experiment (Ref. 52) done at Berkeley records that the larger the rotation speed of the viscometer, the larger the hemolysis found in the blood; this report strongly supports this suggestion. On the other hand, for large-vessel in vivo flow (a non-Newtonian flow), the blood cells flow with almost uniform speeds and concentrate along the axial portion of the vessel. A plasma sheath flowing outside the blood cell column will buffer between the vessel wall and the blood cells. Therefore, the blood cells flowing in large vessels should suffer very little shearing stress and broken damage (Refs. 53 and 54). For small vessel (capillary) flow, the blood cells are bent and squeezed through the vessel with low speed (0.4 mm/s in capillary), but they are not subject to large shearing stress either (Refs. 54 and 55). It is the shearing stress that breaks the blood cells. Following these discussions, it is seen that Dentinfass' experiments actually have many basic differences from the in vivo systems.

### B. Differences Between In Vivo and In Vitro Coagulations

The exact factors causing the differences between the in vivo coagulation and the in vitro coagulation should be carefully differentiated.

The factor of vessel functions has been discussed and weighted in Section I. The factor of flow dilution is analyzed in this report. Now there is another factor that appears, at a first glance, to be responsible for the difference between the two cases of coagulation. This is the sampling activation of the blood. When the in vivo blood is sampled through sampling devices, it may be activated already. But this does not look like the major cause responsible for the difference between the two cases of coagulation. A ph-positive baby subject to total transfusion after birth can still grow normally (without significant thrombosis), although the blood is transfused to his body through an apparatus that may have the same effect as the sampling devices just mentioned (Ref. 56). Therefore, sampling activation cannot be the major factor that makes in vitro blood clot more easily than in vivo blood.

### C. Modification of the Dilution Model, Equation (4)

The present model assumes that the dilution effect is expressed by

$$\frac{\partial M}{\partial t} = -\alpha M \quad (\text{Equation 4})$$

Actually the flow dilution is a very complicated phenomenon. It is a combination of the mechanisms of diffusion, convection, and turbulence. A part of these mechanisms is studied in the fields of chemical transport process and chemical hydrodynamics (Refs. 57-59). It appears that most of the investigators assume that the dilution due to convection-diffusion takes the form of

$$\left(\frac{\partial M}{\partial t}\right)_{c-d} = -\nabla \cdot \bar{J} = -\nabla \cdot (\bar{v}M) = -\bar{v} \cdot \nabla M \quad (19)$$

where  $\bar{v}$  is the statistically averaged stream velocity of the particle, and  $\bar{J}$  is the particle flux due to convection-diffusion. The last equality is valid for incompressible fluid only. On the other hand, the dilution due to turbulence-diffusion is assumed to be

$$\left(\frac{\partial M}{\partial t}\right)_{t-d} = D_t \nabla^2 M \quad (20)$$

where  $D_t$  is a function of the statistical behavior of the eddies in the turbulence. With this new

model of dilution and neglecting the  $\nabla$  and  $\nabla^2$  terms for the intermediates I and Q, we will have a modified following-the-particle rate equation for the thrombin concentration T:

$$\frac{\partial T}{\partial t} = f(T) + D_t \nabla^2 T - \bar{v} \cdot \nabla T \quad (21)$$

where  $f(T) \equiv \theta(\psi - T)$  T is the chemical rate function given by Equation (13).  $\bar{v}$  and  $D_t$  are determined by solving the statistical fluid-dynamic problem encountered. This equation is a nonlinear partial differential equation in space and time. It is very difficult to solve under any realizable boundary and initial conditions. Nevertheless, the Method of Characteristics<sup>6</sup> used in nonlinear fluid dynamics may be promising in application because to a first approximation the

coefficients of the equation do not depend on the independent variables.

It is the author's opinion that this new dilution model may be more accurate than the model assumed in this report. However, in spite of the difficulty of solving Equation (21), the physical effect of this new model on the chemical reactions should be the same as that described in Section IV because the overall effect of the dilution in the new model is still a decrease of the product concentration (T). This decrease will again switch the reaction direction as explained in point 3 of Section IV. Therefore, not too many physical implications can be expected from this new model. Nevertheless, for an accurate quantitative prediction of the critical regulation of in vivo flowing blood, a continuous model like the one given in Equation (21) should be applied.

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<sup>6</sup>Method of Characteristics is a standard method used to solve some nonlinear partial differential equations in hydrodynamics. Examples of the texts dealing with this method are given in Refs. 60 and 61.

## VI. CONCLUSION

This report emphasizes the physical effects, particularly, the flow dilution effect, on the chemical kinetics of blood coagulation *in vivo*. The assumptions are over simplified relative to the extremely complicated picture of *in vivo*

coagulation. Yet, under these simplified assumptions, it is shown rigorously in this report that flow dilution alone can be the factor responsible for the self-regulatory phenomenon of in vivo coagulation.

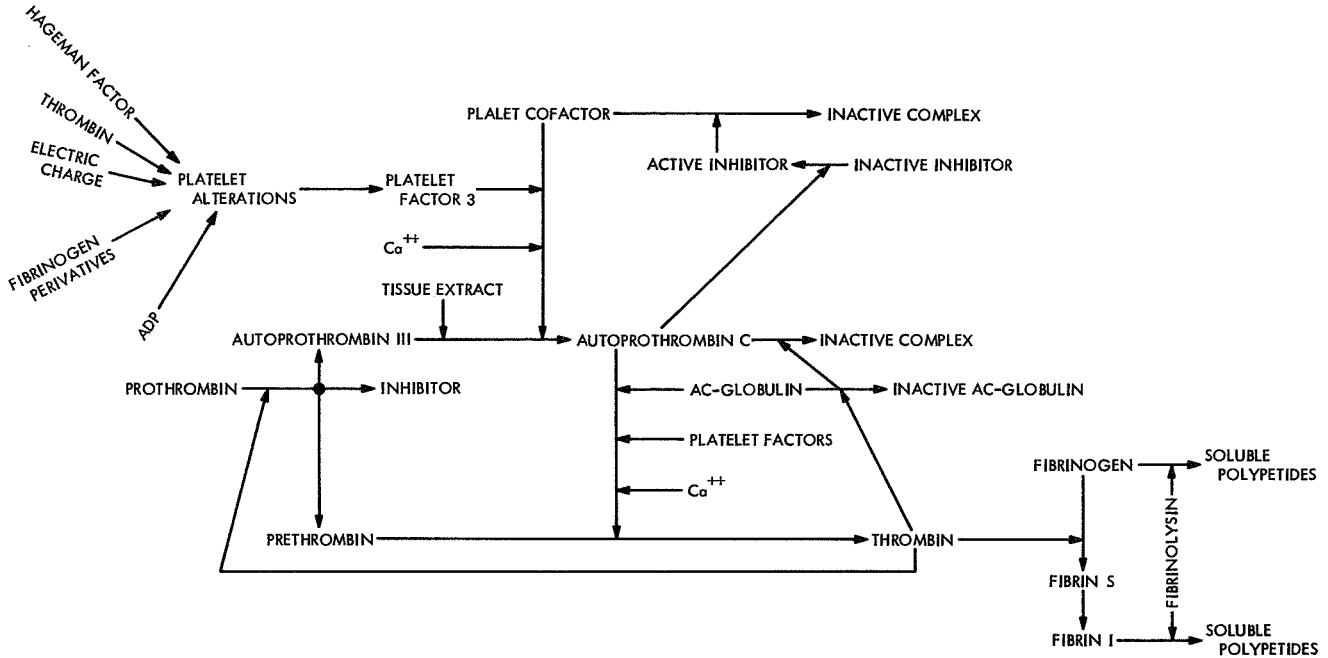


Fig. 1. Seegers' theory of blood clotting (simplified diagram)

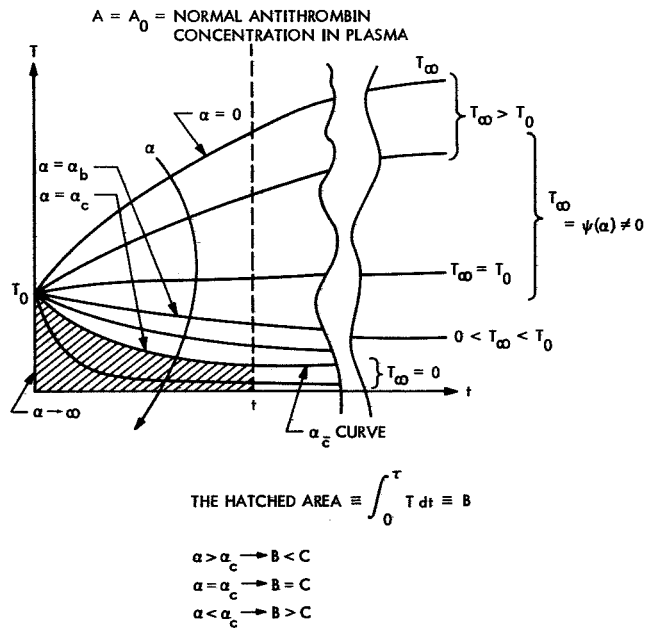


Fig. 2. Variation of thrombin concentration with respect to flow dilution rate

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## APPENDIX A

### VARIATIONS OF T-t CURVES [REPRESENTED BY EQUATION (15) IN THE TEXT] WITH RESPECT TO $\alpha$ OR THE FLOW DILUTION RATE

First, we shall study the variation of T-t curves with respect to  $\theta$  and  $\psi$ . This can be done either by studying the differential Equation (13) or its solution, Equation (15), in the text. The result is shown in Fig. A-1.

Cases 3-1 and 5 have poles at  $t = t_p$  [where the denominator of Equation (15) vanishes]. These cases are not physically realizable. However, as seen in the following, they will never happen for any real values of  $\alpha$  and A, i.e., no real  $\alpha$  and A values will fit all the conditions shown above the sketches of case 3-1 and case 5.

Next, we shall study the variation of  $\theta, \psi$  with respect to  $\alpha$ . Rewriting  $\theta$  and  $\psi$  [Equation (14)] in the following,

$$\theta = \frac{k_1(k_3 - \alpha)}{\alpha + k_2 + k_3}, \quad \psi = P_0 - \left[ \frac{k_4 A}{k_5/(k_6 + \alpha) + 1} + \alpha \right] / \theta \quad (A-1)$$

we see that  $\theta$  decreases monotonically as  $\alpha$  increases, but  $\psi$  decreases monotonically until  $\theta \rightarrow 0$ , then  $\psi$  switches from  $-\infty$  to  $+\infty$  and keeps "positive" when  $\alpha$  increases further. Also multiplying the two expressions in Equation (A-1), we see that  $\theta\psi$  also decreases monotonically as  $\alpha$  increases. Moreover, the asymptotic values of

$\theta, \psi$ , and  $\theta\psi$  as  $\alpha$  approaches infinity are the following:

$$\lim_{\alpha \rightarrow \infty} \theta = -k_1, \quad \lim_{\alpha \rightarrow \infty} \psi = \lim_{\alpha \rightarrow \infty} (-\alpha/\theta) = \infty,$$

$$\lim_{\alpha \rightarrow \infty} \psi\theta = \lim_{\alpha \rightarrow \infty} -\alpha = -\infty$$

Therefore the curves of  $\psi, \theta, \theta\psi$  vs  $\alpha$  must look like that as shown in Fig. A-2.

The last line in the above figure shows the corresponding cases of Fig. A-1 as  $\alpha$  increases.

Finally, we want to prove that the slope of a T-t curve at a fixed T level decreases monotonically as  $\alpha$  increases; i.e., the larger the flow dilution rate, the steeper the T-t curve. This can be shown easily from the differential Equation (13): when T is fixed, the  $\theta\psi$  (Fig. A-2) decreases as the  $\alpha$  increases, hence the less the slope T'. Also as shown in Appendix C, when  $\alpha \rightarrow \infty$ , the T-t curve should approach a stepdown function in time.

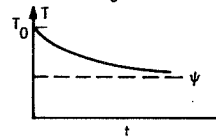
From all the above analyses, we obtain immediately the schematic curves shown in Fig. 2 in the text.



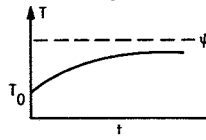
CASE 1. WHEN  $T_0 = 0$ ,  $T = 0$  ALL THE TIME

CASE 2.  $\theta > 0$ ,  $\psi > 0$

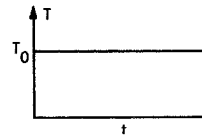
CASE 2-1:  $\psi < T_0$



CASE 2-2:  $\psi > T_0$

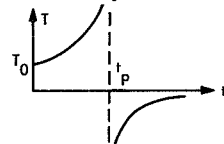


CASE 2-3:  $\psi = T_0$

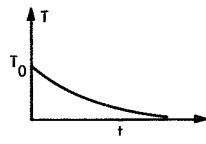


CASE 3.  $\theta < 0$ ,  $\psi > 0$

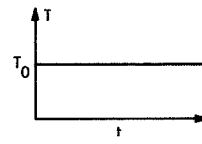
CASE 3-1:  $\psi < T_0$



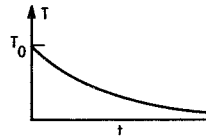
CASE 3-2:  $\psi > T_0$



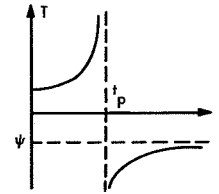
CASE 3-3:  $\psi = T_0$



CASE 4.  $\theta > 0$ ,  $\psi < 0$

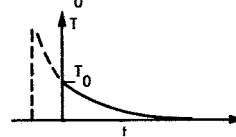


CASE 5.  $\theta < 0$ ,  $\psi < 0$



CASE 6.  $\psi = 0$ ,  $\theta \neq 0$ ,

FROM EQUATION (15),  $T' = -\theta T^2$  AND  $\frac{1}{T} - \frac{1}{T_0} = \theta t$



CASE 7.  $\theta = 0$ ,  $\psi \rightarrow \infty$ , BUT  $\theta\psi = -K$ ,  $K$  IS A POSITIVE CONSTANT. FROM EQUATION (13),

$$T' = -KT \rightarrow T = T_0 e^{-Kt}$$

Fig. A-1. Variation of  $T$ - $t$  curves with respect to  $\theta$  and  $\psi$

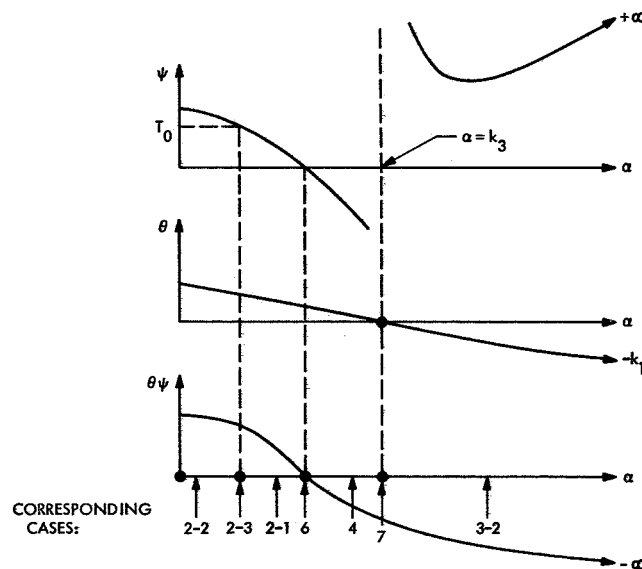


Fig. A-2. Variation of  $\psi$ ,  $\theta$ ,  $\theta\psi$  with respect to flow dilution rate

## APPENDIX B

### VARIATIONS OF T-t CURVES WITH RESPECT TO A

With the result of Fig. A-1 in Appendix A, we need now to study only the variations of  $\theta$ ,  $\psi$  with respect to A when  $\alpha$  is fixed. From Equation (A-1), we see that  $\theta$  is not a function of A, and that  $\psi$  is a monotonically, linearly decreasing function of A if  $\theta$  is positive (i.e., if  $\alpha < k_3$ ), and a monotonically, linearly increasing function of A if  $\theta$  is negative. These variations are shown in Figs. B-1 and B-2, respectively. The last lines in both figures show the corresponding cases in

Fig. A-1 when A increases. It is seen that the variations due to increasing A should be about the same as that due to increasing  $\alpha$ . Similarly, we can prove directly from Equation (13) that the steepness of the T-t curve increases (or slope decreases) as A increases. The schematic drawing of the T-t curves when A varies is omitted here because it is similar to Fig. 2 in the text if all  $\alpha$ 's are replaced by A's.

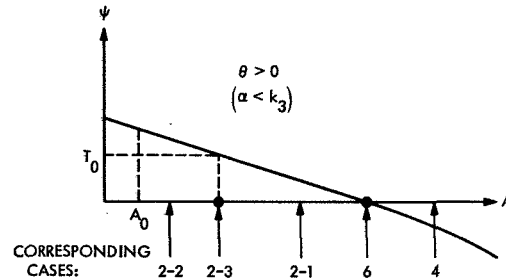


Fig. B-1. Variation of  $\psi$  with respect to antithrombin concentration at low flow dilution

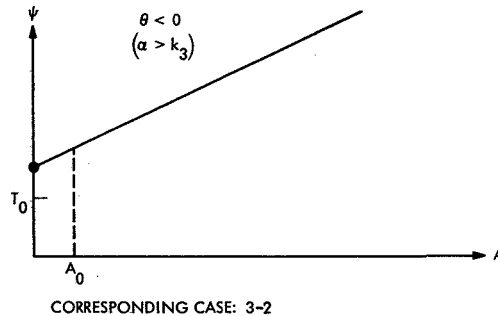


Fig. B-2. Variation of  $\psi$  with respect to antithrombin concentration at high flow dilution

# APPENDIX C

## ASYMPTOTIC T(t) FUNCTION WHEN $\alpha$ IS LARGE

When  $\alpha \gg k_3$ , we see from Fig. A-2 that  $\psi$  will increase to  $+\infty$ . Therefore,  $\psi \gg T_0$  when  $\alpha$  is large, and in the general solution of T, i.e., in Equation (15), we can neglect  $T_0$  as compared with  $\psi$ . Consequently, we have,

$$T \approx T_0 e^{\theta \psi t}$$

when

$$\alpha \gg k_3$$

Since  $\psi > 0$  and  $\theta < 0$  when  $\alpha > k_3$ , this relation is equivalent to

$$T \approx T_0 e^{-|\theta \psi| t}$$

when

$$\alpha \gg k_3 \quad (C-1)$$

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16. Abstract  <p>Self-regulation is a major characteristic pertaining to in vivo blood coagulation. In vivo coagulation is a set of enzyme reactions that are inhomogeneous in space. The inhomogeneity is maintained by the dilution of the blood flow in vivo. Because of this inhomogeneity, the chemical rate equations should be modified by fluid-dynamic (or flow-dilution) change and diffusion change. This report proposes a simple model of enzyme reactions and emphasizes the flow-dilution change of the reactions. First, the complex reactions in blood coagulation are discussed and weighed. Second, two controlling chemical reactions, the prothrombin-to-thrombin conversion and the inactivation of thrombin, both of which have significant positive and negative feedbacks, are selected. It is seen then, that the reaction rates of both these reactions will decrease as thrombin concentration is decreased by flow dilution. But the positive rate decreases more (because of the autocatalyzation of the prothrombin-thrombin conversion) than the negative rate. Therefore when flow dilution increases, the overall reaction direction can be switched from the positive (procoagulative) direction to the negative (anticoagulative) direction; thus the in vivo coagulation is regulated and confined. This physical picture is analytically investigated by solving the modified Michaelis-Menton's enzyme rate equations. The effect of varying the antithrombin concentration is also investigated. The background and the physics of this analysis are extensively discussed.</p>			
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